REMARKS

Reconsideration of the rejections set forth in the Office action mailed April 10, 2006 is respectfully requested. Claims 1-16 are pending in the application. Claims 15-16 are currently withdrawn from consideration.

I. Amendments

Independent claim 1 has been amended to add ordinal letters to the recited steps of the method.

Step (b) of claim 1 has been clarified by changing the phrase "each polynucleotide fragment of the same size ladder having an end and the same oligonucleotide tag as every other polynucleotide fragment of the size ladder" to "each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated". See, for example, page 9 of the specification, second full paragraph, and the illustrations in Figs. 1a-b.

Because the phrase regarding "each polynucleotide fragment having an end" has been removed from step (b) for simplicity, the phrase "the end" in step (d) is changed to "an end".

Dependent claim 4 has been amended to add the phrase "using said polynucleotide as a template". Support is found, for example, at page 8, lines 27-28, and in the description of Figs. 4a-b beginning at page 26, line 29, e.g. at page 27, lines 13-16.

No new matter is added by any of the amendments.

II. Claim Interpretation

The Examiner commented on the phrase "each polynucleotide fragment of the same size ladder having an end and the same oligonucleotide tag as every other polynucleotide fragment of the size ladder" in step (b) of claim 1.

As described, for example, at page 9 of the specification, second full paragraph, fragments of various sizes are generated for each of the tag-polynucleotide conjugates recited in step (a) of the claim, and each fragment retains the same oligonucleotide tag as the original full size tag-polynucleotide conjugate. This is also illustrated schematically in Fig. 1a. The claim language has also been amended along these lines, as noted above.

(Regarding the Examiner's comment as to "multiple identical copies of each polynucleotide", the original population of tag-polynucleotide conjugates, represented at 100 in Fig. 1a, could certainly include multiple identical copies of each polynucleotide, and typically would, as long as "substantially every different polynucleotide has a different oligonucleotide tag attached", as required by the claim.)

III. Rejections under 35 U.S.C. §102(b)

Claim 1 and dependent claims 4, 6-8 and 10 were rejected under 35 U.S.C. §102(b) as being anticipated by Wong (U.S. Patent No. 5,935,793). This rejection is respectfully traversed for the following reasons.

For a prior art reference to anticipate in terms of 35 U.S.C. §102, every element of the claimed invention must be identically shown in a single reference. ...The elements must be arranged as in the claim under review... *In re Bond*, 910 F.2d 831, 15 USPQ2d 1566 (Fed. Cir. 1990); MPEP §2131.

A. The Invention

Independent claim 1 recites a method of simultaneously determining a signature sequence for each polynucleotide in a population of polynucleotides, the method comprising the steps of:

(a) attaching an oligonucleotide tag from a repertoire of tags to each polynucleotide of the population to form tag-polynucleotide conjugates, such that substantially every different polynucleotide has a different oligonucleotide tag attached;

(b) generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate, each polynucleotide fragment having the same oligonucleotide tag as the tag-polynucleotide conjugate from which it was generated;

(c) separating the polynucleotide fragments into size classes;

(d) labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides at an end of such polynucleotide fragment;

(e) copying the labeled oligonucleotide tags[1] of each polynucleotide fragment of each

[1] Applicants note that, on pages 3 and 6 of the Office Action, the Examiner has misquoted step (d) of the claim as "labeling the *polynucleotide*", rather than "labeling the oligonucleotide tag".

size class; and

(f) separately hybridizing the labeled oligonucleotide tags of each size class with their respective complements under stringent hybridization conditions, the respective complements being attached as populations of substantially identical oligonucleotides in spatially discrete and addressable regions on one or more solid phase supports, and the respective signature sequences being determined by the sequence of labels associated with each spatially discrete and addressable region of the one or more solid phase supports.

The role of "size ladders" and "size classes" in the claimed method

In accordance with the invention (see e.g. the embodiment of Fig. 1b), prior to sequencing the target polynucleotides, each is processed into a size ladder (step b above). The size ladder for a given polynucleotide contains different-length portions of the sequence of that polynucleotide, each also containing the tag that corresponds to the original polynucleotide.

These size ladders for the population of polynucleotides are combined, and the resulting mixture of tagged fragments is separated into size classes (step c above). A size class will therefore contain differently tagged fragments from different polynucleotides, but the fragments within a class will be of a similar length, simplifying subsequent processing and sequencing of the fragments.

B. The Prior Art

Wong describes a sequencing method in which a population of polynucleotides to be sequenced are provided with "identifier tags". The tagged polynucleotides are then processed according to conventional sequencing methods, typically by Sanger sequencing or Maxam-Gilbert sequencing (see column 17, lines 3-9).

In the Maxam-Gilbert approach, as known in the art, the polynucleotide to be sequenced is cleaved specifically at a given nucleotide, for each of the four different nucleotides, generating a population of different-length fragments for each nucleotide. In the Sanger approach, as also known in the art, a polynucleotide to be sequenced is used as a template for replication, and a given terminating nucleotide, such as a dideoxynucleotide, is used to generate a series of different-length fragments terminating in that nucleotide.

The different-length fragments so produced are separated, typically by electrophoresis. In the method of Wong, each fragment can then be correlated with the polynucleotide from which it is derived, by way of the above-noted "identifier tags". Thus, a population of polynucleotides can be sequenced simultaneously on the same electrophoresis medium.

Labeling of species in Wong

Maxam-Gilbert sequencing is discussed in Wong at column 14, lines 23-53. As stated therein, the polynucleotides may be labeled, "for subsequent detection in the array hybridization step", prior to chemical degradation.

Sanger sequencing is preferred by Wong and is discussed, for example, at column 12, line 57 to column 13, line 62. As stated therein, "primer extension reactions may be conducted together using a single aliquot of the vector mixture if four different labels attached to the 3'-terminator bases are used to distinguish the terminating base-types. Alternatively, when a four-label method is used, wherein the labels are carried on the extension primer, the primer extension reactions may be separately conducted in four different aliquots, one for each base-type, which upon completion may be combined for all subsequent processing steps."

Wong does not teach the method of claim 1

The applicants' method differs from that taught by Wong, in one aspect, in that the steps of "(b) generating a size ladder of polynucleotide fragments" and "(c) separating the polynucleotide fragments into size classes", in the applicants' claim, both precede the step of "(d) labeling the oligonucleotide tag of each polynucleotide fragment according to the identity of one or more nucleotides...". That is, the fragments are separated into size classes before sequencing or labeling takes place (see e.g. Fig. 1b).

As described in the excerpts above from Wong, the step of "generating a size ladder of polynucleotide fragments" (i.e. by Sanger sequencing or Maxam-Gilbert sequencing) follows, or is concomitant with, labeling (of the extension primer or the terminating nucleotide in Sanger sequencing, or the uncleaved starting polynucleotide in Maxam-Gilbert sequencing). The step of "separating the polynucleotide fragments into size classes" in Wong (i.e. separating the Sanger or Maxam-Gilbert sequencing fragments by

electrophoresis) also follows the step of labeling in Wong.[2]

In view of the above comments, the reference does not disclose all of the elements set out above in claim 1, and arranged as in this claim. Claim 1 and its dependent claims therefore cannot be anticipated by this reference, and the applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §102(b).

IV. Rejections under 35 U.S.C. §103

Claim 1 and dependent claims 2-12 and 14 were rejected under 35 U.S.C. §103(a) as being unpatentable over Brenner (U.S. Patent No. 5,763,175) in view of Wong, cited above. The rejections are respectfully traversed in light of the following remarks.

A. The Invention

The invention, as embodied in independent claim 1, is discussed above in Section IIIA.

B. The Cited Art

Brenner is directed to a method of simultaneous sequencing of polynucleotides. As shown in the embodiment of Fig. 2, multiple sets of "S primers" are applied to a construct made up of an S primer binding site (22), the target polynucleotide (20), a tag (16) flanked by cleavage sites (14 and 18), and a second primer binding site (12). The S primers are provided in sets of four, as shown in the Figure, where within each set one nucleotide is either A, G, C, or T. The different sets of S primers (shown as Sets 1, 2, 3, ...k) differ in how far this varying nucleotide, which is the site of sequence interrogation, extends into the target polynucleotide.

As described e.g. at column 13, lines 14-26 and column 20, lines 26-55 of the patent, separate PCR reactions are carried out with the different primer sets. For a given target

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[2] One exception is described at column 22, lines 13-22 of Wong, where "primer-tag-primer" regions of separated fragments are labeled during PCR amplification, "to allow ready detection of the amplified tag sequences". However, the label in this case is the same for every amplified species, contrary to the applicants' claim, which recites "labeling the oligonucleotide tag of each polynucleotide fragment *according to the identity of one or more nucleotides at an end of such polynucleotide fragment*".

polynucleotide, only one primer of each set of four, i.e. the one that matches the target polynucleotide at the varying nucleotide, results in amplification.

The tag of each amplicon can then be labeled according to which of the four nucleotides was present in the primer (see e.g. column 13, lines 35-45). The labeled tags are then cleaved and hybridized to an array of tag complements, and the label (and thus the detected nucleotide) is detected for each position on the array.

The S primers in Brenner also contain a IIS restriction enzyme recognition site, so that, after a round of sequencing as described above, the end of the polynucleotide which has been sequenced can be cleaved off. See, for example, the set of primers at column 20, lines 5-15 of the patent. Because the position of the recognition site is constant with respect to the primer binding site, the enzyme will cleave at the same position in the target polynucleotide for each primer.

The Examiner's characterization of the reference

Contrary to the Examiners' statement at (b1) on page 6 of the Office Action, Brenner does not teach "generating a size ladder of polynucleotide fragments for each tag-polynucleotide conjugate" (step b of applicants' claim 1). As explained above, the enzymatic cleavage from the IIS recognition site in each S primer (using BbvI in the working Example) will cleave at the same position in the target polynucleotide for each primer, and thus will not create a "size ladder". For the same reason, this shortening does not encompass "shortening by a different amount said polynucleotides of said tag-polynucleotide conjugates in each aliquot such that said polynucleotides in different aliquots are shortened a different amount" (Examiner's statement at (b2), regarding dependent claim 2).

Even if this shortening did form a "size ladder" of some sort, the process would bear no relation in context to the generation of a size ladder in step (b) of applicants' claim. In Brenner, this shortening is carried out after a round of sequencing, so that the end of the polynucleotide which has been sequenced can be cleaved off: "In the shortening cycles, a predetermined number of previously identified nucleotides are cleaved from the target polynucleotides and the shortened polynucleotides are employed in the next cycle of nucleotide identification" (column 5, lines 6-12; see also column 14, lines 31-43). In the

applicants' method, on the contrary, the size ladder is formed from each tag-polynucleotide conjugate before any labeling (step d) or sequence determination (steps d-f) takes place.

Moreover, the Examiner's statement at (b3) regarding ligation of the S primers in Brenner is not pertinent to dependent claim 4, which recites "forming extension products of known lengths for each tag-polynucleotide, using said polynucleotide as a template".

Nor does Brenner show "copying the labeled oligonucleotide tags of each polynucleotide fragment" (step e of applicants' claim 1), as asserted on page 6 of the Office Action, since in Brenner, the tags are labeled after they have been copied (i.e. selectively amplified) (see e.g. column 13, lines 35-45 and column 21, lines 23-28).

C. Analysis

In order to establish a *prima facie* case of obviousness, there must be some suggestion or motivation, either in the references or in knowledge generally available to one skilled in the art, to modify a reference or combine reference teachings. The prior art must also provide a reasonable expectation of success. Finally, the prior art reference, or references when combined, must teach or suggest all the claim limitations. (MPEP §2143)

As discussed above, a distinguishing feature of applicants' claimed method is that tagged polynucleotides to be sequenced are processed into size ladders, and the resulting tagged fragments are then separated into size classes, before sequencing of the fragments and labeling of the oligonucleotide tags takes place. Such a feature is shown in neither of the cited references.

In fact, Brenner, as discussed above, does not teach forming a "size ladder" from the target polynucleotides at all. The only "size ladders" formed in the Wong patent constitute Sanger or Maxam-Gilbert sequencing fragments, to be later separated by electrophoresis. The sequencing strategy employed in Brenner (extension of specifically hybridized primers) is mechanistically quite different from Sanger or Maxam-Gilbert sequencing; Sanger sequencing is discussed in the Background of Brenner as a conventional but inefficient sequencing technique. Therefore, there would be no reason to form such sequencing fragments in the method of Brenner.

In view of the foregoing, the claimed method includes features taught by neither cited reference; motivation is lacking to combine the teachings of Wong with those of Brenner; and, even if this were done, the results would not teach or suggest the applicants' claimed method. Accordingly, the applicants respectfully request the Examiner to withdraw the rejection under 35 U.S.C. §103(a).

V. Conclusion

In view of the foregoing, the applicant submits that the claims now pending are now in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

Respectfully submitted,

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